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Bacillus Anthrax Real Time PCR Kit User Manual

For In Vitro Diagnostic Use Only

Σ 25 −20 °C IVD

REF **ZD-0073-01**

For use with LightCycler2.0 Instrument

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1. Intended Use

The bacillus anthrax real time PCR Kit is a test for the detection of bacillus anthrax in swab, biopsy or serum sample by using the real time PCR systems.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Anthrax is primarily a disease of herbivorous mammals, although other mammals and some birds have been known to contract it. There are 3 types of anthrax in humans: cutaneous anthrax, gastrointestinal tract anthrax and pulmonary (inhalation) anthrax. The cutaneous form accounts for 95% or more of human cases globally. All 3 types of anthrax are potentially fatal if not treated promptly. The bacillus anthracis real time PCR Kit contains a specific ready-to-use system for the detection of the bacillus anthracis using PCR (polymerase chain reaction) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the bacillus anthracis DNA. Fluorescence is emitted and measured by the real time systems´ optical unit during the PCR. The detection of amplified bacillus anthracis DNA fragment is performed in fluorimeter **channel 530nm** with the fluorescent quencher BHQ1. DNA extraction buffer is available in the kit and swab, biopsy or serum samples are used for the extraction of the DNA. In addition, the kit contains a system to identify possible PCR inhibition by measuring the 560nm fluorescence of the internal control (IC).

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	2 vials, 1.5ml
2	ROB Reaction Mix	1 vial, 480µl
3	PAG Reaction Mix	1 vial, 480µl
4	CAPA Reaction Mix	1 vial, 480µl
5	PCR Enzyme Mix	1 vial, 32µl
6	Molecular Grade Water	1 vial, 400ul
7	Internal Control (IC)	1 vial, 30µl
8	ROB Positive Control	1 vial, 30µl
9	PAG/CAPA Positive Control	1 vial, 60µl

Analysis sensitivity: 1×10^4 copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and Freezer
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)

7. ⚠Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Avoid aerosols.

8. Sample Collection, Storage and transport

- Collected samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

9.1.1 Swab or biopsy sample

1) Wash the sample in 0.5ml normal saline and vortex vigorously. Centrifuge at 13000rpm for 2 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 100µl DNA extraction buffer to the tube, closed the tube then vortex for 10 seconds.
3) Incubation the tube for 10 minutes at 100°C.
4) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the extracted DNA and can be used for the template of the PCR.

9.1.2 Serum sample

1) Pipet 50µl sample to a new 0.5ml tube, add 50µl DNA extraction buffer, closed the tube then vortex for 10 seconds.
2) Incubation the tube for 10 minutes at 100°C.
3) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the extracted DNA and can be used for the template of the PCR.

Attention:

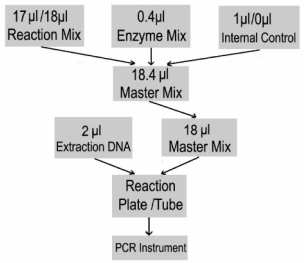
A. During the incubation, make sure the tube is not open. Since the vapor will volatilize into the air and may cause contamination if the sample is positive.
B. The extraction sample should used in 3 hours or store at -20°C for one month.
C. DNA Extraction kits are available from various manufacturers. The customer can use their own extraction systems or the commercial kit depending on the yield. Please carry out the RNA extraction according to the manufacturer's instructions if use a RNA extraction kit.

9.2 Internal Control

It is necessary to add internal control (IC) in **ROB** reaction mix. Internal control (IC) allows the user to determine and control the possibility of PCR inhibition.
Add the internal control (IC) 1µl/rxn and the result will be shown in the 560nm Channel.

9.3 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



※PCR system without HEX/VIC/JOE channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample.

Reaction Volume	ROB Master Mix Volume	CAPA/PAG Master Mix Volume
Reaction Mix	17µl × (n+1)	18µl × (n+1)
Enzyme Mix	0.4µl × (n+1)	0.4µl × (n+1)
Internal control (IC)	1µl × (n+1)	—

Mix completely then spin down briefly in a centrifuge.

2) Pipet **18 µl Master Mix** using micropipets with sterile filter tips to each of the real time PCR reaction plate/tubes. Add **2µl** of the DNA sample or positive/ negative controls to each of the plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:

37°C for 2min	1cycle
94°C for 2min	1cycle
93°C for 5sec, 60°C for 30sec (Fluorescence measured at 60°C)	40cycles

Selection of fluorescence channels	
530nm	Target Nucleic Acid
560nm	IC

10. Threshold setting: Choose **Arithmetic** as back ground and **none** as Noise Band method, then adjust the Noise band just above the maximum level of molecular grade water, and adjust the threshold just under the minimum of the positive control.

11. Quality control: Negative control, positive control, internal control must be performed correctly, otherwise the sample results is invalid.

Control \ Channel	Crossing point value	
	Channel 530nm of 3 kinds of Reaction Mix	Channel 560nm of ROB Reaction Mix
Molecular Grade Water	Blank	25~35
Positive Control(qualitative assay)	≤35	——

12. Data Analysis and Interpretation

The following results are possible:

	Crossing point value		Result Analysis
	530nm	560nm	
1#	Blank	25~35	Below the detection limit or negative
2#	≤38	——	Positive
3#	38～40	25~35	Re-test; if it is still 38~40, report as 1#
4#	Blank	Blank	PCR Inhibition; no diagnosis can be concluded.

13. Data Analysis and Interpretation

	Crossing point value		Result Analysis
	Channel 530nm of 3 kinds of Reaction Mix	Channel 560nm of ROB Reaction Mix	
1#	Blank	25~35	Negative "—"
2#	≤38	——	Positive "+"
3#	38～40	25~35	Re-test; If it is still 38~40, report as 1#
4#	Blank	Blank	PCR Inhibition; No diagnosis can be concluded.

Channel	530nm			560nm	Result Analysis
Reaction Mix	ROB	PAG	CAPA	ROB	
1#	–	–	–	25~35(crossing point vaule)	Below the detection limit or negative
2#	+	+	+	——	high virulent
3#	+	+	–	——	low virulent
4#	+	–	+	——	low virulent
5#	+	–	–	——	avirulent

For further questions or problems, please contact our technical support at **trade@liferiver.com.cn**